Variation in the Airborne Fungal Spore Population of the Tuscarawas Valley with Respect to Microenvironment, Time of Day, and Date[1]

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ABSTRACT. **Quantitative and qualitative viable airborne fungal spore counts were made from samples collected at five sites, four times per day on 1, 8, 24, and 30 June, 1988 at the Tuscarawas Campus of Kent State University. Significant variation among quantitative samples was evident from nonoverlapping standard errors of the means. Lawn, hay field, and soybean field locations had significantly higher counts than parking lot and stream bank microenvironments. The highest counts occurred during the morning, when many molds disperse their spores in response to warming temperatures and the concurrent reduction in relative humidity. During early June, when rainfall was more abundant, the counts were significantly higher than during late June. The total June qualitative count included 73.5% *Cladosporium* spp., 13.4% brightly colored mycelia sterilia, 6.9% *Alternaria* spp. and 6.2% other molds. The chi-square test for independence confirms that significant variation exists among microenvironments, at different times of day, on different dates.**

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INTRODUCTION

Airborne fungal spore populations are of interest to mycologists, plant pathologists, population biologists, and allergists. The peculiarities of fungal spore populations are especially important for predicting risk of respiratory allergic reactions in sensitized individuals or the possibility for developing opportunistic mycoses in immunocompromised persons. Recent studies in the United States have determined variation among outdoor viable airborne fungal spore populations, but do not emphasize the dynamics of the microenvironment (Levetin and Horowitz 1978, Al-Doory et al. 1980, 1982). Consequently, this study examines the variation associated with outdoor microenvironments, and it documents the dynamic effect of the microenvironment, time of day, and date on the viable airborne fungal spore count.

The data presented here are part of a study of the airborne fungi of the Tuscarawas Valley. The results from collections made at five locations on the Kent State University Tuscarawas Campus during June 1988 are included. Locations were widely separated from one another and had sufficiently distinctive features to constitute separate microenvironments. These sites, which are typical of outdoor environments in eastern Ohio, include a large lawn of mixed grasses and weedy plants without trees or buildings, an asphalt-paved parking lot, a hay field, a soybean field, and a tree-lined stream bank.

MATERIALS AND METHODS

A portable platform was carried among the designated sites to collect airborne fungal spore samples. Its aluminum foil-covered horizontal surface (15 cm x 25 cm) was elevated 80 cm above the ground, and its cover was replaced with new foil prior to each sample collecting cycle. The foil surface was disinfected with 0.5% glutaraldehyde prior to collecting individual samples. To collect a sample, two Petri plates with a yeast-malt-agar (YMA) medium were opened simultaneously for three min on the platform. This provided a collecting surface of 110.8 cm^2. The medium was prepared by dissolving 0.5 g yeast extract, 10.0 g malt extract, and 15.0 g agar in one litre of boiling water and then autoclaving the mixture.

Below the sampling platform surface were a thermometer and a hygrometer for determining temperature and relative humidity. A compass and a wind meter were used to determine wind speed and direction. In conjunction with each sample collection, notes on ground cover, surface moisture, cloud cover, ecological activity, and recent weather conditions were recorded.

The sample plates were transported to and from the collection sites as packages of two plates in a plastic bag. The packages of plates were maintained in a horizontal, noninverted position in stacking racks. Upon return to the laboratory, the plates were incubated at 23° C within the plastic bags.

Plates were examined and quantitative colony counts were made on the sixth day of incubation. At that time colonies were developed and could be described, but had not appreciably grown together. Each colony observed on the sixth day was assumed to have originated from a single spore. Plate counts were converted to hourly deposit rates per 110.8 cm^2 by multiplying the combined counts of the two plates in the sample by 20. Qualitative plate counts, during which genera were identified, were delayed to the seventh or eighth day of incubation to give fungi fertile on this medium additional time to sporulate. Colonies were first examined at 100 X magnification to observe the arrangement of conidia on conidiophores. Colony fragments mounted in lactophenol cotton blue were then examined for conidial morphology at 430 X magnification.

Statistical tests used to determine significant differences in sample data were nonoverlapping standard errors of the means for quantitative counts and the chi-square test for independence with qualitative counts (Ambrose and Ambrose 1987, Zar 1984). The five sites in the study were sampled four times per day on 1, 8, 24, and 30 June. Data collected on 16 June were disregarded

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TABLE 1

Morning Spore Counts at the K. S. U. Tuscarawas Campus During June 1988.

Date	Lawn		Parking Lot		Hay Field		Stream Bank		Bean Field	
	Count	AI	Count	AI	Count	AI	Count	AI	Count	AI
1 June	1320	0.51	260	0.10	7240	2.80	420	0.16	1160	0.45
8 June	2840	1.10	360	0.14	3020	1.17	520	0.20	2160	0.83
24 June	3740	1.44	320	0.12	160	0.06	360	0.14	200	0.08
30 June	2460	0.95	80	0.03	140	0.05	40	0.02	80	0.03
Mean	2590	1.00	255	0.10	2549	1.02	335	0.13	900	0.35

Count: Viable spores deposited per 110.8 cm^2 per hour.

AI: Abundance Index, count compared to mean morning lawn count.

TABLE 2

Mid-day Spore Counts at the K. S. U. Tuscarawas Campus During June 1988.

Date	Lawn		Parking Lot		Hay Field		Stream Bank		Bean Field	
	Count	AI	Count	AI	Count	AI	Count	AI	Count	AI
1 June	860	0.33	220	0.08	600	0.23	560	0.22	3620	1.26
8 June	860	0.33	180	0.07	1700	0.66	360	0.14	320	0.12
24 June	300	0.12	80	0.03	180	0.07	220	0.08	80	0.03
30 June	280	0.11	220	0.08	200	0.08	160	0.06	40	0.02
Mean	575	0.22	175	0.07	670	0.26	325	0.13	1015	0.39

Count: Viable spores deposited per 110.8 cm^2 per hour.

AI: Abundance Index, count compared to mean morning lawn count.

TABLE 3

Afternoon Spore Counts at the K. S. U. Tuscarawas Campus During June 1988.

Date	Lawn		Parking Lot		Hay Field		Stream Bank		Bean Field	
	Count	AI	Count	AI	Count	AI	Count	AI	Count	AI
1 June	460	0.18	240	0.09	740	0.29	140	0.05	440	0.17
8 June	460	0.18	1600	0.61	1700	0.66	500	0.19	540	0.21
24 June	300	0.12	20	0.01	180	0.07	60	0.02	80	0.03
30 June	380	0.15	360	0.14	260	0.10	120	0.05	180	0.07
Mean	400	0.15	555	0.21	720	0.28	205	0.08	310	0.12

Count: Viable spores deposited per 110.8 cm^2 per hour.

AI: Abundance Index, count compared to mean morning lawn count.

TABLE 4

Evening Spore Counts at the K. S. U. Tuscarawas Campus During June 1988.

Date	Lawn		Parking Lot		Hay Field		Stream Bank		Bean Field	
	Count	AI	Count	AI	Count	AI	Count	AI	Count	AI
1 June	320	0.12	140	0.05	2760	1.07	540	0.21	1020	0.39
8 June	4040	1.56	420	0.16	980	0.38	380	0.15	320	0.12
24 June	840	0.32	240	0.09	180	0.07	580	0.22	140	0.05
30 June	120	0.07	20	0.01	140	0.05	80	0.05	280	0.11
Mean	1330	0.51	205	0.08	1015	0.39	395	0.15	440	0.17

Count: Viable spores deposited per 110.8 cm^2 per hour.

AI: Abundance Index, count compared to mean morning lawn count.

because rain showers contaminated several plates. While individual plates received exposures of three min duration, a complete collection cycle required two hr in order for the investigator to move the sample collection apparatus sequentially from site to site. Morning samples were collected between 7:30 AM and 9:30 AM, mid-day samples between 11:30 AM and 1:30 PM, afternoon samples between 3:30 PM and 5:30 PM, and evening samples between 7:30 PM and 9:30 PM.

RESULTS

QUANTITATIVE COUNTS. The Tuscarawas Campus is characterized by extensive heterogenous lawns of mixed grasses and weedy broadleaved plants with isolated groves of deciduous and evergreen trees. Initial collections suggested that the lawns were major consistent contributors to the airborne fungal spore populations of the campus. Therefore, for ease in comparing the data from the several sites and their separate collections, the June 1988 mean morning spore count from the unshaded lawn has been used as a standard index equal to 1.00. All other sample counts have been converted to percentages of this standard index.

The morning samples (Table 1) taken at the five sites during the four weekly collections of June 1988 had the following standardized mean spore count indices: lawn 1.00, parking lot 0.10, hay field 1.02, stream bank 0.13, and soybean field 0.35. The mid-day collections (Table 2) had the following standardized mean indices: lawn 0.22, parking lot 0.07, hay field 0.26, stream bank 0.13, and soybean field 0.39. The afternoon standardized mean indices were: lawn 0.15, parking lot 0.21, hay field 0.28, stream bank 0.08, and soybean field 0.12 (Table 3). From the evening collections (Table 4) standardized mean indices were: lawn 0.51, parking lot 0.08, hay field 0.39, stream bank 0.15, and soybean field 0.17.

The significance of the variation observed in the quantitative counts from samples collected at different microenvironments at different times of day and on different dates is evident from the nonoverlapping standard errors of their means, which indicates significant difference with 68.26% confidence (Zar 1984). Among the five microenvironments, the airborne fungal spore populations of the lawn, hay field, and soybean field were significantly greater than the spore populations of the parking lot and stream bank (Table 5A, Fig. 1). A comparison of spore counts from different times of day reveals that the morning counts were significantly greater than those obtained at other times. The lowest daily values tended to occur during the afternoon, but they did not differ appreciably from mid-day or evening data (Table 5B, Fig. 1). Variation in the magnitude of the spore populations sampled on different dates is also evident. The spore counts obtained on 1 and 8 June were substantially higher than those from 24 and 30 June (Table 5C, Fig. 1).

QUALITATIVE COUNTS. The relative abundance of different types of fungi in the collections from June 1988 shows great variation throughout the month (Tables 6, 7, 8 and 9). The predominant hyphomycetes observed were

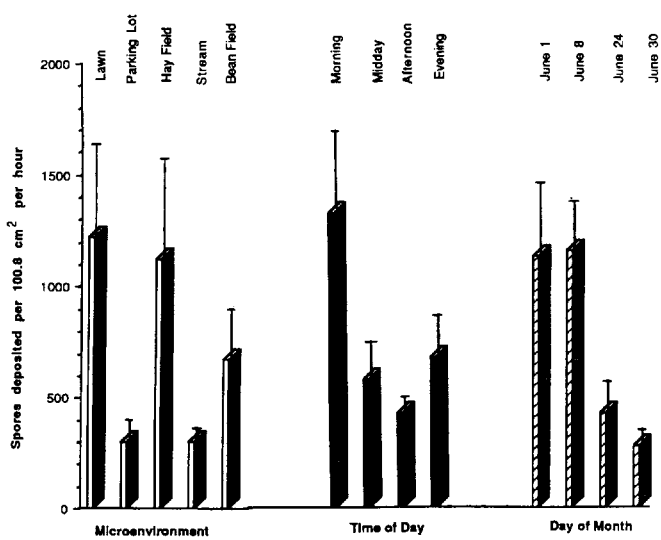

FIGURE 1. Comparison of influence of microenvironment, time of day, and day of the month on rate of deposition of airborne fungal spores. Bars represent the mean ±SE for each condition.

TABLE 5

Statistical summary of variation among samples from June 1988 airborne fungal spore populations at the Tuscarawas Campus.

A. Microenvironment Variation (combined dates and times of day)

	Lawn	P. Lot	Hay Field	Stream	Bean Field
Range	120-4040	20-1600	140-7240	40-580	40-3620
Mean (AI)	1224 (0.47)	298 (0.11)	1121 (0.49)	315 (0.12)	666 (0.26)
S. E.	325	92	464	49	241

B. Time of Day Variation (combined dates and microenvironments)

	Morning	Mid-day	Afternoon	Evening
Range	40-7240	40-3620	20-1700	20-4040
Mean (AI)	1344 (0.52)	552 (0.21)	433 (0.17)	677 (0.26)
S. E.	405	183	102	223

C. Date Variation (combined microenvironments and times of day)

	1 June	8 June	24 June	30 June
Range	140-7240	180-4040	20-3740	20-2460
Mean (AI)	1153 (0.45)	1163 (0.45)	413 (0.16)	277 (0.11)
S. E.	376	246	180	117

AI = Abundance Index

TABLE 6

Qualitative spore counts (no./110.8 cm²/3 min.) for 1 June 1988.

Time/Site	*Cladosporium*	Mycelia Sterilia	*Alternaria*	Other Molds	Total (%)
Morning/Lawn	50	8	1	7	66 (12.7)
P. Lot	8	5	0	0	13 (2.5)
Hay Field	311	33	18	0	362 (69.6)
Stream	14	4	1	2	21 (4.0)
Bean Field	38	16	2	2	58 (11.2)
Total (%)	421 (81.0)	66 (12.7)	22 (4.2)	11 (2.1)	520 (100.0)
Mid-day/Lawn	26	8	7	2	43 (14.7)
P. Lot	4	3	3	1	11 (3.8)
Hay Field	22	1	4	3	30 (10.2)
Stream	19	2	1	6	28 (9.6)
Bean Field	113	8	34	26	181 (61.8)
Total (%)	184 (62.8)	22 (7.5)	49 (16.7)	38 (13.0)	293 (100.0)
Afternoon/Lawn	11	9	0	3	23 (22.8)
P. Lot	10	1	1	0	12 (11.9)
Hay Field	28	0	2	7	37 (36.6)
Stream	2	1	2	2	7 (6.9)
Bean Field	17	4	1	0	22 (21.8)
Total (%)	68 (67.3)	15 (14.9)	6 (5.9)	12 (11.9)	101 (100.0)
Evening/Lawn	16	0	0	0	16
P. Lot	5	0	1	1	7 (2.9)
Hay Field	78	46	11	3	138 (57.7)
Stream	14	7	2	4	27 (11.3)
Bean Field	36	10	0	5	51 (21.3)
Total (%)	149 (62.3)	63 (26.4)	14 (5.9)	13 (5.0)	239 (100.0)
Day Total (%)	822 (71.3)	166 (14.4)	91 (7.9)	74 (6.4)	1153 (100.0)

TABLE 7

Qualitative spore counts (no./110.8 cm²/3 min.) for 8 June 1988.

Time/Site	*Cladosporium*	Mycelia Sterilia	*Alternaria*	Other Molds	Total (%)
Morning/Lawn	133	8	0	1	142 (31.9)
P. Lot	12	4	0	2	18 (4.0)
Hay Field	125	8	10	8	151 (33.9)
Stream	17	3	4	2	26 (5.8)
Bean Field	97	3	3	5	108 (23.4)
Total (%)	384 (86.3)	26 (5.8)	17 (3.8)	18 (4.0)	445 (99.9)
Mid-day/Lawn	32	9	2	0	43 (25.1)
P. Lot	9	0	0	0	9 (5.3)
Hay Field	80	1	1	3	85 (49.7)
Stream	12	2	1	3	18 (10.5)
Bean Field	11	1	1	3	16 (9.4)
Total (%)	144 (84.2)	13 (7.6)	5 (2.9)	9 (5.3)	171 (100.0)
Afternoon/Lawn	19	1	1	2	23 (9.6)
P. Lot	68	6	4	2	80 (33.3)
Hay Field	82	0	1	2	85 (35.4)
Stream	15	6	1	3	25 (10.4)
Bean Field	18	1	3	5	27 (11.3)
Total (%)	202 (84.2)	14 (5.8)	10 (4.2)	14 (5.8)	240 (100.0)
Evening/Lawn	162	28	11	1	202 (65.8)
P. Lot	14	2	1	4	21 (6.8)
Hay Field	41	3	3	2	46 (16.0)
Stream	12	0	0	7	19 (6.2)
Bean Field	11	3	0	2	16 (5.2)
Total (%)	240 (78.2)	36 (11.7)	15 (4.9)	16 (5.2)	307 (100.0)
Day Total (%)	970 (83.4)	89 (7.7)	47 (4.0)	57 (4.9)	1163 (100.0)

Cladosporium spp., which accounted for 73.5% of the month's total collection. The greatest frequency of *Cladosporium* (86.3%) occurred during the morning collection on 8 June, and the lowest frequency (43.8%) occurred on 30 June during the evening collection. The second most frequently observed type of mold was a moderately fast-growing, bright-colored sterile mycelium with yellow-orange pigmentation often tinged with pink or tan. It accounted for 13.4% of the month's total collection and was most abundant on 30 June (32.0%) in the morning collection. This peculiar mold was least prominent on 8 June, when it accounted for 5.8% of the morning and afternoon collections. The third most abundant type of mold was *Alternaria* spp. Their greatest frequency was 18.6% of the 24 June mid-day collection, whereas their lowest frequency was 2.9% of the 8 June mid-day collection. Overall, *Alternaria* spp. accounted for 6.9% of the June 1988 collection. Various other molds were observed with overall frequencies less than 1.0%. These included dark-colored mycelia sterilia, hyaline or subhyaline mycelia sterilia, pycnidial molds, pseudomycelial yeasts, and species of *Epicoccum, Drechslera, Penicillium, Aspergillus, Verticillium, Cunninghamella,* and *Circinella*. Red and white yeast colonies were often observed in the culture plates, but their numbers are not included in the percentages reported above.

The significance of the variation in qualitative colony counts produced by samples collected from the various microenvironments was determined by subjecting the total counts for June 1988 from each microenvironment to the chi-square test for independence (Table 10). The chi-square value of 133.743, with 12 degrees of freedom, far exceeds the 0.001 probability critical value and is, therefore, very highly significant. This supports the effect of the microenvironment on the qualitative sample counts. Upon analysis of the contingency table used to compute this chi-square value, most of the deviation resulted from the molds in the less common group being more abundant than expected at the stream bank and the soybean field, and less abundant than expected at the lawn and hay field. The bright-colored sterile mycelia were more abundant than expected at the lawn, but less abundant than expected in the hay field. *Alternaria* spp. were more abundant than expected in the soybean field, while *Cladosporium* spp. were somewhat greater in the hay field and somewhat lesser than expected at the stream bank.

The effect of the time of day on the qualitative colony counts is evident from the range of percentages within the different fungal groups at the different collection times.

TABLE 8

Qualitative spore counts (no. /110.8 cm²/3 min.) for 24 June 1988.

Time/Site	*Cladosporium*	Mycelia Sterilia	*Alternaria*	Other Molds	Total (%)
Morning/Lawn	147	31	7	2	187 (78.2)
P. Lot	13	2	1	0	16 (6.7)
Hay Field	7	0	1	0	8 (3.3)
Stream	12	1	2	3	18 (7.5)
Bean Field	6	2	2	0	10 (4.2)
Total (%)	185 (77.4)	36 (15.1)	13 (5.4)	5 (2.1)	239 (100.0)
Mid-day/Lawn	8	2	5	0	15 (34.9)
P. Lot	1	1	1	1	4 (9.3)
Hay Field	7	0	2	0	9 (20.9)
Stream	6	4	0	1	11 (25.6)
Bean Field	2	1	0	1	4 (9.3)
Total (%)	24 (55.8)	8 (18.6)	8 (18.6)	3 (7.0)	43 (100.0)
Afternoon/Lawn	9	5	1	0	15 (46.9)
P. Lot	0	0	1	0	1 (3.1)
Hay Field	2	2	2	3	9 (28.1)
Stream	2	1	0	0	3 (9.4)
Bean Field	1	1	0	2	4 (12.5)
Total (%)	14 (43.8)	9 (28.1)	4 (12.5)	5 (15.6)	32 (100.0)
Evening/Lawn	23	15	2	2	42 (42.4)
P. Lot	3	5	3	1	12 (12.1)
Hay Field	5	1	2	1	9 (9.1)
Stream	17	1	8	3	29 (29.3)
Bean Field	5	0	2	0	7 (7.1)
Total (%)	53 (53.5)	22 (22.2)	17 (17.2)	7 (7.1)	99 (100.0)
Day Total (%)	276 (66.8)	75 (18.2)	42 (10.2)	20 (4.8)	413 (100.0)

The significance of this variation was determined from a contingency table (Table 11) where chi-square is 77.477. This value, with nine degrees of freedom, has an associated probability far less than 0.001, making the effect of the time of day on the qualitative count very highly significant. The major contributors to the deviation include fewer than expected bright-colored mycelia sterilia at mid-day, but greater than expected numbers of these fungi during the evening. *Alternaria* spp. are more prevalent at mid-day than expected, but they are fewer than expected during the morning. The other molds category was more abundant than expected at mid-day, while being less abundant than expected during the morning. The percentages of *Cladosporium* spp. were least affected by the time of day.

Variation in the qualitative counts among the different collection dates is evident from the wide range of percentages among the fungal groups observed. The significance of this variation (Table 12) is revealed by a chi-square value of 146.911, which has an associated probability much less than 0.001. Among the factors producing the deviation are *Cladosporium* spp. recorded with greater than expected frequency on 8 June, but with less than expected frequency on 30 June. In contrast, bright-colored mycelia sterilia were fewer than expected on 8 June, but greater than expected on 30 June. *Alternaria* counts were subnormal on 8 June, whereas the other molds group count was elevated on 30 June.

When the chi-square test for independence was applied to the data from the separate collection cycles of each of the four sampling days using 0.05 probability for the critical value, all four collections on 1 June and 8 June showed significant variation in the qualitative distribution of the four groups of fungi among the various microenvironments. On 24 June, only the morning and evening collections showed significant variation in distribution, and on 30 June none of the collections varied significantly.

DISCUSSION

EFFECT OF MICROENVIRONMENT. Because most fungi are saprophytes or parasites of plant tissues, the vegetation in a given area is a major influence on the quantity and quality of airborne fungal spores. In grain-growing areas, one would expect spores of rust and smut fungi in great abundance, whereas in forested areas spores of Basidiomycetes would be plentiful (Bulmer 1969). These principles have guided studies where the airborne fungal spore population of a generalized macroenvironment has been the major concern of the investigators (Levetin and Horowitz 1978, Al-Doory et al. 1980). However, while addressing the question of uniformity of airspora concentrations, Kramer and Eversmeyer (1987) found that when

TABLE 9

Qualitative spore counts (no./110.8 cm²/3 min.) for 30 June 1988.

Time/Site	*Cladosporium*	Mycelia Sterilia	*Alternaria*	Other Molds	Total (%)
Morning/Lawn	85	53	16	10	164 (90.6)
P. Lot	3	1	0	0	4 (2.2)
Hay Field	3	2	0	2	7 (3.9)
Stream	1	0	0	1	2 (1.1)
Bean Field	1	2	1	0	4 (2.2)
Total (%)	93 (51.4)	58 (32.0)	17 (9.4)	13 (7.2)	181 (100.0)
Mid-day/Lawn	6	2	3	3	14 (31.1)
P. Lot	8	1	2	0	11 (24.4)
Hay Field	7	2	1	0	10 (22.2)
Stream	5	0	1	2	8 (17.8)
Bean Field	1	1	0	0	2 (4.4)
Total (%)	27 (60.0)	6 (13.3)	7 (15.6)	5 (11.1)	45 (100.0)
Afternoon/Lawn	18	4	0	0	22 (32.4)
P. Lot	12	2	0	4	18 (26.5)
Hay Field	6	2	2	3	13 (19.1)
Stream	3	2	1	0	6 (8.8)
Bean Field	4	1	1	3	9 (13.2)
Total (%)	43 (63.2)	11 (16.2)	4 (5.9)	10 (14.7)	68 (100.0)
Evening/Lawn	2	1	1	2	6 (18.8)
P. Lot	0	0	0	1	1 (3.1)
Hay Field	3	1	0	3	7 (21.9)
Stream	1	0	0	3	4 (12.5)
Bean Field	8	3	2	1	14 (43.8)
Total (%)	14 (43.8)	5 (15.6)	3 (9.4)	10 (31.3)	32 (100.1)
Day Total (%)	177 (54.3)	80 (24.5)	31 (9.5)	38 (11.7)	326 (100.0)

TABLE 10

Contingency table for the chi-square test for independence for the relative abundance of major airborne fungi at different microenvironments (KSU-Tuscarawas, June 1988).

		Sites					
Fungal group		Lawn	Parking lot	Hay field	Stream bank	Bean field	Total
Cladosporium	o	747	170	807	152	369	2,245
	e	751.763	174.897	741.475	185.185	391.681	
M.S. bright	o	184	33	102	34	57	410
	e	137.293	31.941	135.414	33.820	71.532	
Alternaria	o	57	18	60	24	52	211
	e	70.656	16.438	69.689	17.405	36.813	
Other molds	o	35	17	40	42	55	189
	e	63.289	14.724	62.423	15.590	32.974	
Total		1,023	238	1,009	252	533	3,055

$$\frac{(o-e)^2}{e} =$$

0.030 + 0.137 + 5.791 + 5.947 + 1.313 +
15.890 + 0.035 + 8.245 + 0.001 + 2.952 +
2.639 + 0.148 + 1.347 + 2.499 + 6.265 +
12.645 + 0.352 + 8.055 + 44.739 + 14.713 = X^2

$X^2 = 133.743$; d.f. = 12; $p<<0.001$

TABLE 11

Contingency table for the chi-square test for independence for the relative abundance of major airborne fungi at different times of day. (KSU-Tuscarawas, June 1988).

Fungal group		Time of Day: Morning	Mid-day	Afternoon	Evening	Total
Cladosporium	o	1,083	379	327	456	2,245
	e	1,017.782	405.643	324.074	497.501	
M. S. bright	o	186	49	49	126	410
	e	185.876	74.082	59.185	90.858	
Alternaria	o	69	69	24	49	211
	e	95.658	38.125	30.459	46.758	
Other molds	o	47	55	41	46	189
	e	85.684	34.150	27.283	41.883	
Total		1,385	552	441	677	3,055

$\frac{(o-e)^2}{e}$ =

4.179 + 1.750 + 0.026 + 3.462 +
0.000 + 8.492 + 1.753 + 13.592 +
7.429 + 25.004 + 1.370 + 0.108 +
17.465 + 12.730 + 6.896 + 0.405 = X^2

X^2 = 77.477; d.f. = 9; p<<0.001

TABLE 12

Contingency table for the chi-square test for independence for the relative abundance of major airborne fungi on different dates (KSU-Tuscarawas, June 1988).

Fungal group		Date: June 1	June 8	June 24	June 30	Total
Cladosporium	o	822	970	276	177	2,245
	e	847.295	854.643	303.498	239.565	
M. S. bright	o	166	89	75	80	410
	e	154.740	156.082	55.427	43.751	
Alternaria	o	91	47	42	31	211
	e	79.634	80.325	28.525	22.516	
Other molds	o	74	57	20	38	189
	e	71.331	71.950	25.551	20.168	
Total		1,153	1,163	413	326	3,055

$\frac{(o-e)^2}{e}$ =

0.755 + 15.571 + 2.491 + 16.340 +
0.819 + 28.831 + 6.912 + 30.033 +
1.622 + 13.826 + 6.365 + 3.197 +
0.100 + 3.106 + 1.206 + 15.767 = X^2

X^2 = 146.911; d.f. = 9; p<<0.001

moisture was adequate, spore counts such as from *Cladosporium* spp. were significantly higher at 1.5 m than at 9 m or 30 m over grass plots, and that dilution in horizontal distribution of rust spores occurred downwind from their source of production. In the present study, attention has been given to the effect which the microenvironment produces both quantitatively and qualitatively on airborne fungal spore samples. Although localized areas of profuse production have their spores distributed by the wind to contribute to the spore counts at other sites,

the distribution is not accomplished instantaneously. Hence, the composition of the airborne fungal spore population of a generalized region is not homogeneous. The significant differences recorded from the Tuscarawas Valley emphasize the unique contributions of each microenvironment. Whereas the lawn and pre-harvest hay field contributed copious amounts of *Cladosporium* spp. and bright-colored mycelia sterilia, the stream bank, with its herbaceous, arboreal, and aquatic features, contributed the highest percentage of less common molds. From the earliest days of the study of mycological physiology, investigators recognized that moisture and nutrients are essential to the growth of fungi, and that fungal growth is a prerequisite to sporulation (Lilly and Barnett 1951). It follows that large numbers of airborne fungal spores should be associated with locations favoring fungal growth. Thus, the lawn with its humus substrata formed by accumulated decomposing grass and herbaceous plant clippings, which are sheltered from desiccation by the lawn's new growth, is ideal for fungus growth and maturation. The pre-harvest hay field provides a similar environment that is very conducive to the growth and maturation of molds. By comparison, the asphalt-covered parking lot is without vegetation, and it dries easily on hot sunny days. Few spores are produced in the parking lot itself. Most of its spore count is caused by airborne spores carried by the wind from adjacent areas. The low count at the stream bank may have occurred because of a robust growth of herbaceous plants on the banks and the arboreal canopy having only recently expanded. This healthy young plant growth had not yet been colonized by fungi. In addition, the trees of the stream banks provide a windbreak which acts as a screen to reduce the entrance of airborne spores from adjacent fields. The moderate spore counts of the soybean field may be attributed to decomposing vegetation in the soil from the previous season's crop.

Effect of Time of Day. Morning is the time of day when spore maturation and dispersal occurs for many molds. During the morning, light intensity and temperature are increasing with a concurrent decrease in relative humidity while the morning dew evaporates (Ingold 1965). Morning breezes assist the dispersal of mature spores. Under mid-day and afternoon conditions of higher temperature, lower relative humidity, and more intense solar radiation, fewer viable spores mature. During the evening, lower temperatures and increased relative humidity restore conditions favorable to spore maturation and dispersal. Royes (1987) reported a study of the major airborne fungi of Jamaica during 1968 and 1969. *Cladosporium* was the predominant airborne fungus, and diurnal periodicity in spore dispersal was associated with rising morning temperatures accompanied by reduced relative humidity. In the present study from the Tuscarawas Valley, spore counts from the lawn consistently followed the typical diurnal pattern. The highest counts (1.00) occurred during morning. At mid-day they were reduced (0.22), and they reached their lowest levels (0.15) during the afternoon. Under evening conditions, spore counts over the lawn begin to increase (0.51). Because the parking lot produces few spores itself, it serves as a background monitor or a type of control in experiments in which the dynamics of microenvironments are being evaluated. The morning spore count at the parking lot compared to the standard index was 0.10, at mid-day it was 0.07, during the afternoon it was 0.21, and during the evening it was 0.08. The elevated afternoon count deserves an explanation. On the afternoon of 8 June, the hay field adjacent to the parking lot was being mowed. The machinery moving through the field produced noticeable clouds of dust that were carried towards the parking lot by northeasterly breezes. If the uncharacteristic sample (0.68) from that afternoon is disregarded, then the afternoon mean index (0.09) differs little from mean sample values for morning, mid-day and evening, suggesting an absence of significant diurnal fluctuation in the airborne fungal spore population associated with the parking lot. The standard mean indices for June 1988 from the hay field also suggest diurnal fluctuations in the spore counts. Morning produced the highest counts (1.02), the mid-day value (0.26) is slightly less than the afternoon value (0.28), while evening values are elevated (0.39). The mean quantitative counts from the stream bank do not suggest significant diurnal fluctuation. The standard indices were 0.13 at morning and mid-day, 0.08 during the afternoon, and 0.15 during the evening. The seclusion of this microenvironment and its more varied fungal flora are thought to account for the stability in quantitative counts. Fluctuations in the mean morning (0.35), mid-day (0.39), afternoon (0.12), and evening (0.17) indices recorded from the soybean field reflect variation in wind and soil conditions associated with date rather than fluctuations strictly associated with time of day, as explained below. Yet, if the uncharacteristic value for mid-day on 1 June is disregarded, then a diurnal pattern is evident with its lowest value (0.06) occurring at mid-day.

Effect of Date. Variation in the quality and quantity of airborne fungal spores is not surprising in view of the multitude of dynamic factors which interact to affect fungal growth and sporulation. Bulmer (1969) identifies season, temperature, wind and weather along with vegetation, soil composition, and diurnal periodicity. Royes (1987) noted the importance of rainfall for localized spore production in Jamaica. While studying vertical variation in airborne spore concentrations over Kansas turf grass and grain crop research plots, Lyon, Kramer, and Eversmeyer (1984) found that during 1977, when rainfall was 157% of normal, *Cladosporium* counts at 1.5 m differed significantly from those at 9 m or 30 m heights. During 1978, when rainfall was below normal, no significant variation occurred among the three heights. In the Kansas study, *Alternaria* was significantly more abundant at 1.5 m heights than at 9 m or 30 m during short periods of dry weather. In the present study from the Tuscarawas Valley, the effects of daily conditions and seasonal change on airborne fungal spore populations are also evident. The differences in the quantitative counts between the first two weeks and the last two weeks of June 1988 were created by diminished rainfall. As a continuum of hot dry days during late June developed into drought, fungal

growth and sporulation was not as prolific as during early June. Another way in which the date affects studies in spore population is through human activity which alters collection sites. The hay field was mowed midway during the June 1988 study, and under drought conditions no new growth of its grasses occurred. Prior to hay harvest, diurnal fluctuations in spore counts were the most extreme among the microenvironments in the study, and the hay field was the most prolific contributor to the airborne spore population of the campus. Following hay harvest, the spore counts from the hay field differed little from the general background, as measured at the parking lot. In the soybean field, soil preparation for planting on 1 June created a loose powdery soil surface from which gusty winds formed clouds of airborne dust during the early June collections. Rain showers on 16 June solidified the surface, and in the subsequent drought it baked into a brick-like cover around stunted soybean plants, which may have prevented spores of soil fungi from entering the air.

Interpreting Qualitative Data. This study has focused on assessing variation among airborne fungal spore samples collected from diverse microenvironments of eastern Ohio, but it also provided a foundation for reporting the mycological flora of the region, a project of greater scope which the investigator continues to pursue. The limited number of genera reported at this time reflects the brief duration of this segment of the project, as well as the unfavorable conditions resulting from the drought in Ohio during the summer of 1988. Comparisons of qualitative data with other recent studies of outdoor airborne molds in the United States are complicated because investigators use a variety of sampling methods and different ways of reporting generic frequencies. Yet, some genera appear to be cosmopolitan and present in abundance. In a one-year study of the outdoor mold from Tulsa, OK (Levetin and Horowitz 1978), the predominant genera were *Hormodendrum* (*Cladosporium*), *Alternaria, Pullularia* (*Aureobasidium*), *Epicoccum, Helminthosporium Drechslera*), sterile mycelia, *Penicillium, Papularia,* and *Phoma*. In studies from the Washington, DC, metropolitan area (Al-Doory et al. 1980, 1982) the major genera were *Hormodendrum* (*Cladosporium*), *Aureobasidium, Alternaria, Phoma, Penicillium, Epicoccum, Fusarium,* and *Aspergillus*.

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